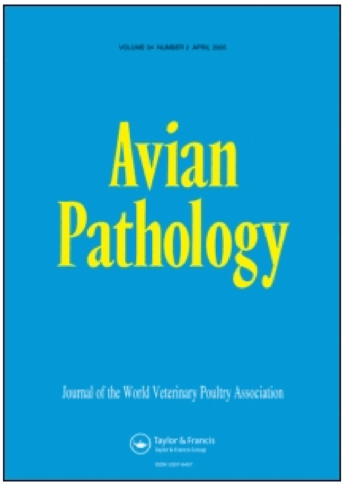

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Experimental trichothecene mycotoxicosis produced in broiler chickens by Fusarium sporotrichiella var. sporotrichioides

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EXPERIMENTAL TRICHOTHECENE MYCOTOXICOSIS PRODUCED IN BROILER CHICKENS BY *FUSARIUM SPOROTRICHIELLA* VAR. *SPOROTRICHIOIDES*

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SUMMARY

Fusarium sporotrichiella var. *sporotrichioides* (Bilay), cultured on sterilised popcorn at 23°C and then at 8°C, 16°C and 23°C and fed as 50% of the diet, was lethal to 7-day-old male broiler chickens. The 8°C culture, containing T-2 toxin at 50 parts per million (ppm) and neosolaniol at 5 ppm, was given as whole culture at dietary concentrations of 10%, 5%, 1% and 0% for 17 days and 1% for 42 days. Half the chickens that were fed the 10% diet died during the 17 days (5 ppm T-2 toxin and 0.5 ppm neosolaniol). The corresponding daily dose was 0.24 mg T-2 toxin and 0.02 mg neosolaniol/kg body weight/day. The chickens that died were dehydrated, had necrosis and depletion of lymphoid and haematopoietic tissues and necrosis of the hepatobiliary system, gastroenteric mucosa, feather epidermis and renal tubular epithelium. The survivors had anaemia, reduction of weight gain and transiently altered righting reflex. The comb and beak were pale yellow and the feather barbs were dishevelled. Survivors also had atrophied lymphoid tissues, reduced haematopoietic cellularity in the bone marrow, necrosis of oral and crop mucosa, vacuolated hepatocytes, hyperplastic bile ductules, and reduction of the thyroid follicular diameter.

INTRODUCTION

Trichothecene mycotoxins are among the metabolites produced on cereal grains and forages by *Fusarium*. Of these, T-2 toxin, diacetoxyscirpenol, deoxynivalenol

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and nivalenol occur alone or in combinations in feedstuffs (Ghosal *et al.*, 1978; Greenway and Puls, 1976; Mirocha *et al.*, 1976; Jemmali *et al.*, 1978; Siegfried, 1977; Vesonder and Ciegler, 1979).

As a group, but with individual variation in potency, trichothecenes are contact irritants and are either radiomimetic or cytotoxic toxins (Saito *et al.*, 1969; Ueno, 1977). Avian fusariotoxicosis is considered to be caused by trichothecenes because field and experimental forms of the disease involve ulceration of the oral mucosa. T-2 toxin was a cause of avian fusariotoxicosis in the United States (Wyatt *et al.*, 1972), Canada (Greenway and Puls, 1976) and France (Renault *et al.*, 1979). Avian fusariotoxicosis occurred in Russia in association with human alimentary toxic aleukia (Kurmanov and Novacky, 1978).

In this study, a Hungarian isolate of *Fusarium* was cultured on corn and fed to broiler chickens. Specific toxins were identified in the cultures, their concentrations were measured, the lethal dietary concentrations of the cultures and dose were defined, and the clinical signs and gross and microscopic lesions were described.

MATERIALS AND METHODS

Fungi

Fusarium sporotrichiella var. *sporotrichioides* MC72073 (Bilay, also *F. sporotrichioides* and *F. tricinctum*) was isolated from wheat associated with a mycotoxicosis of chickens in Hungary. Popcorn (280 g) was mixed with water (28 ml) in 1 1 flasks which were stoppered with cotton and sterilised at 121°C for 45 min. After inoculation, the incubation schedules were: 23°C for 4 weeks, 23°C for 2 weeks followed by 16°C for 2 weeks, and 23°C for 1 week followed by 8°C for 4 weeks. The cultures were then treated with propionic acid (1%), dried at 38°C, treated again with propionic acid (0.5%) and stored at 4°C.

Mycotoxin analysis

(a) Trichothecenes. Culture substrate (100 g) was extracted twice with $CH_3OH{:}H_2O$ (300 ml, 40:60) in a blender for 3 min. Methanol was evaporated in a forced-air oven and the water was removed by freeze-drying. Each freeze-dried residue was extracted twice with methanol (150 ml) in a blender for 3 min. Methanol extracts from each freeze-dried residue were combined and dried. The methanol solubles were chromatographed on silica gel presaturated with $CHCl_3$. The column was eluted consecutively with $CHCl_3$ (100 ml), $CHCl_3{:}CH_3OH$ (95:5; 250 ml) and CH_3OH (100 ml). Each eluate was monitored for trichothecenes by thin-layer chromatography (TLC) (Vesonder *et al.*, 1977). Residue from the $CHCl_3{:}CH_3OH$ eluate was further analysed by gas chromatography-mass spectrometry by converting to its trimethysilyl (TMS) ether derivative (Vesonder *et al.*, 1978). Analyses were made by direct comparison with TMS derivatives of authentic T-2 toxin, vomitoxin, nivalenol, fusarenone, monoacetoxyscirpenol, neosolaniol and diacetoxyscirpenol. Quantitation of the TMS derivatives of trichotehcenes was by the method of Vesonder *et al.* (1977) on a Finnigan Gas Chromatograph-Mass Spectrometer. The column was 3% OV-1 on Gas Chrom (6 ft x 2 mm), and was temperature programmed from 160°C to 250°C at increments of 5°C per min.

(b) 4-acetamido-4-hydroxy-2-butenoic acid-γ lactone (butenolide). A chick starter feed of which 50% was whole fungal culture was extracted twice with dichloromethane [CH_2Cl_2] (500 ml) in a Waring blender. Extracts were combined, dried with a rotary evaporator, and oil-like residue [A] was spotted on a silica gel plate and analysed for

butenolide by TLC with the developing solvent, toluene:ethyl acetate (1:3) (Yates *et al.*, 1970). The TLC plate was sprayed with 2,4-dinitrophenylhydrazine reagent and heated at 100°C for 3 to 5 min for detection of butenolide. The infrared (IR) spectrum of [A] in CH_2Cl_2 solution was compared with that of authentic butenolide. In addition, the CH_2Cl_2 extraction was extracted twice with methanol in a blender for 3 min. The extracts were combined, dried with a rotary evaporator and the residue was extracted with ether and filtered. Ether solubles [B] and ether insolubles [C] were analysed for butenolide by TLC and IR as for residue [A].

Chickens
Male broiler chickens (Hubbard x Hubbard strain) were obtained at 1-day-old from a commercial hatchery and were acclimated until 7-days-old. Wire-floored cages were heated to 35°C to 40°C for 14 days and to 25°C thereafter.

Toxicology
The feeding trials (Table 1) used 7-day-old chickens. Experimental diets comprised inactivated fungal cultures mixed with nonmedicated chick starter mash. Control chickens were fed starter mash only. Feed and water were given *ad libitum* and feed consumption was measured on alternate days. The microhaematocrit was measured on trial days 0, 7, 17, 37 and 42. The righting reflex, feathers, and oral mucosae were examined weekly and general clinical condition was evaluated daily.

Table 1. Treatment groups and deaths of chickens fed Fusarium sporotrichiella *corn cultures.*

Trial	Culture conditions (°C)	Diet (% culture)	Days	Deaths per group
1	23°. 8°	50%	7	9/10
	23°, 16°	50%	7	2/5
	23°	50%	7	2/5
1A	23°, 8°	25%	7	8/10
2	23°, 8°	10%	17	5/10
	23°, 8°	5%	17	0/10
	23°, 8°	1%[a]	17	0/10
		0%[a]	17	0/10
2A	23°, 8°	1%	42	0/8
		0%	42	0/8

a Eight chickens from each of these groups were continued on study 2A.

Pathology
All chickens were necropsied; survivors were killed with CO_2 gas. The spleen, bursa of Fabricius, and liver and gallbladder (together) from survivors were blotted dry and weighed. Tissues were fixed in neutral-buffered 10% formalin, processed by routine histological techniques and stained with haematoxylin and eosin. Selected tissues from the mouth were stained by the periodic acid-Schiff (PAS) procedure. Selected livers were stained by PAS, and frozen sections from formalin-fixed liver were stained with oil red O.

The body weight, organ weights and haematocrit were analysed for homogeneity of variance within the groups prior to analysis of variance (Anderson and McLean, 1974). The absolute organ weight (Stevens, 1976) was analysed for variance with the initial body weight as a covariate (Shirley, 1977). The Duncan and Student-Newman-Keuls (SNK) multiple range tests were used to evaluate effects that varied as to dose (Nie *et al.*, 1975).

RESULTS

Mycotoxin analyses

TLC of the $CHCl_3$:CH_3OH eluate revealed spots in the Rf regions of T-2 toxin and neosolaniol. Analysis of the TMS ether derivatives by GC:MS revealed three trichothecenes: T-2 toxin, neosolaniol and diacetoxyscirpenol (Table 2, toxin recovery $\geqslant 85\%$).

Butenolide was not detected in the oil-like residue [A] by TLC, nor did the IR spectrum of [A] show the band 1790 and 1760^{-1} characteristic for butenolide in CH_2Cl_2 solution. A similar analysis on [B] and [C] did not detect butenolide. The detection concentration of this procedure was 25 ppm.

Table 2. Trichothecene mycotoxins produced by Fusarium sporotrichiella *cultured at 8°, 16°* and 23°C.

Culture temperature	Parts per million		
	T-2 toxin	Neosolaniol	Diacetoxyscirpenol
23°, 8°	50	5	None detected
23°, 16°	123	15	2.4
23°	147	44	None detected

Toxicology

In Trial 1 and 1A, consumption of 50% or 25% 8°*Fusarium* culture caused a rapid decrease in spontaneous activity and most of the chickens died within 24 hours. A smaller percentage died and the deaths occurred later in the groups that were fed *Fusarium* cultured at 16°C or 23°C. The 8°C *Fusarium* culture was the most toxic and was used in Trial 2.

Five died in a group of 10 chickens that were fed 10% *Fusarium* culture for 17 days (Trial 2). The lethal concentration for T-2 toxin, based upon dilution of the culture, was 5 ppm and for neosolaniol, 0.5 ppm. The corresponding lethal dose of 0.24 mg T-2 toxin and 0.02 mg neosolaniol/kg body weight/day was calculated from the average daily feed consumption.

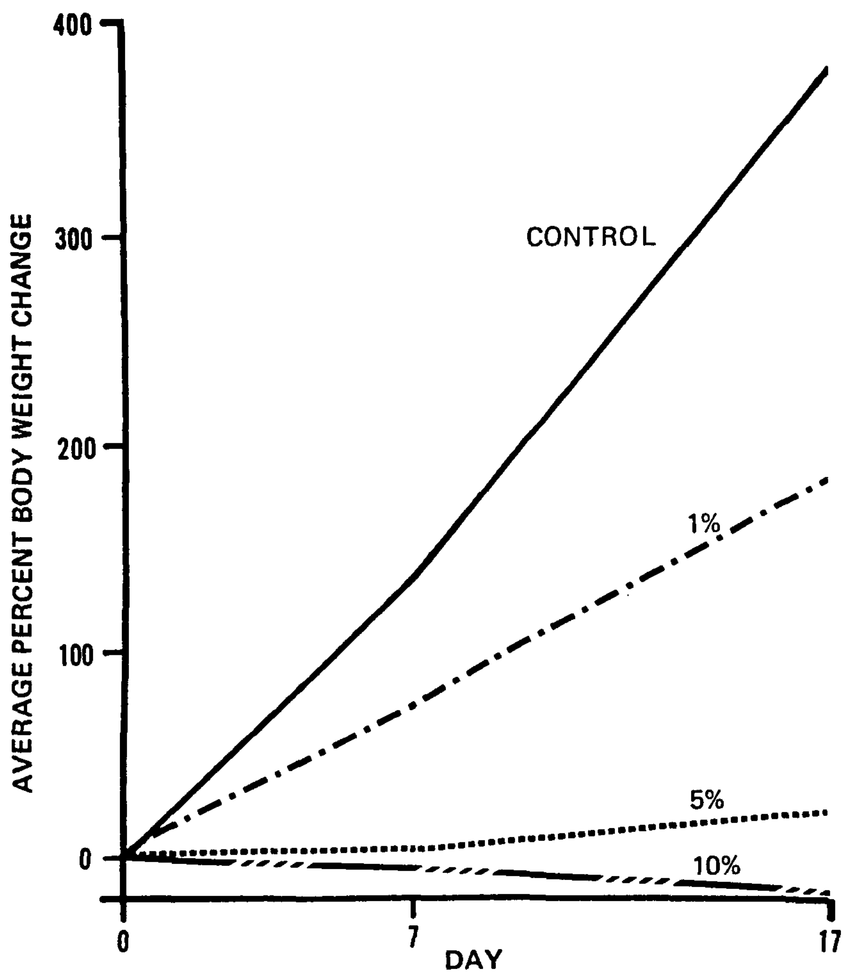
Increased concentrations of fungal culture in the diet caused greater reductions in feed consumption (Table 3). The average daily dose correlated positively with the fungal culture concentration during the 17-day trial. Chickens that were continued on a diet of *Fusarium* culture for 42 days, however, eventually tolerated a larger average daily dose of trichothecenes than those fed 1% or 5% *Fusarium* for 17 days.

The body weight of chickens that died within 24 hours was reduced 10%. In survivors, the 10% *Fusarium* diet caused a weight reduction and the 5% *Fusarium* diet allowed for a slight increase in weight (Test-fig.1). The "no effect concentration" for weight gain was less than 1% *Fusarium* culture and a corresponding T-2 toxin concentration of 0.5 ppm, and for neosolaniol, 0.05 ppm (Table 4).

Table 3. Average daily feed consumption and calculated toxin exposure of chickens fed Fusarium sporotrichiella *corn cultures.*

Diet % fungal culture	Trial days	Feed consumption (g/chicken/day)	(mg/kg mean body wt/day)	
			T-2 toxin	Neosolaniol
10%	17	4.3	0.24	0.024
5%	17	8.6	0.18	0.018
1%	17	31.8	0.12	0.012
1%	42	53.4	0.20	0.02
0%	42	62.0	0[a]	0[a]

a Control diet was not analysed for trichothecenes.



Text-fig. 1. Average percent body weight change of chickens fed Fusarium *diets for 17 days.*

Table 4. Organ weight and final body weight values of chickens fed Fusarium sporotrichiella *corn cultures.*

Diet % fungal culture	Trial days	Spleen	Bursa of Fabricius	Liver	Body
		(g) ($\bar{x}$ ± S.E.) Range			
10%	17	0.03 ± 0.01 0.02 – 0.04	0.05 ± 0.012 0.02 – 0.06	4.5 ± 0.4 2.6 – 4.6	100 ± 17 85 – 122
5%	17	0.08 ± 0.03 0.02 – 0.14	0.10 ± 0.05 0.03 – 0.18	6.2 ± 0.8 3.1 – 11.2	128 ± 36 65 – 170
1%	17	0.16 ± 0.04 0.13 – 0.19	0.94 ± 0.46 0.61 – 1.27	12.2 ± 0.8 11.4 – 13	326 ± 52 290 – 363
0%	17	0.55 ± 0.21 0.40 – 0.70	2.54 ± 0.27 2.35 – 2.74	32.7 ± 0.8 31.9 – 33.5	591 ± 65 545 – 638
1%	42	0.57 ± 0.37 0.22 – 1.25	3.25 ± 2.23 0.23 – 6.30	26.6 ± 1.7 19.7 – 33.8	1007 ± 193 758 – 1255
0%	42	2.22 ± 0.47 1.33 – 2.8	6.29 ± 1.95 4.0 – 9.75	38.5 ± 1.9 33.4 – 50	1732 ± 136 1435 – 1884

The righting reflex was consistently abnormal in five chickens given 5% *Fusarium* culture and one given 1% *Fusarium* culture. Others in the 1% group were affected intermittently but all were normal on day 42. Abnormal righting reflex was not associated with low haematocrit values.

Pathology

The group mean haematocrit decreased progressively in chickens fed 1% or 5% *Fusarium* culture (Table 5). The five survivors fed 10% culture group, however, had a group mean haematocrit similar to controls. Heterogeneity within groups precluded analysis of variance. Four patterns of haematocrit values occurred in individual chickens: normal throughout the treatment period, transient decrease followed by an increase to normal, prolonged moderated decrease, and progressive severe decrease.

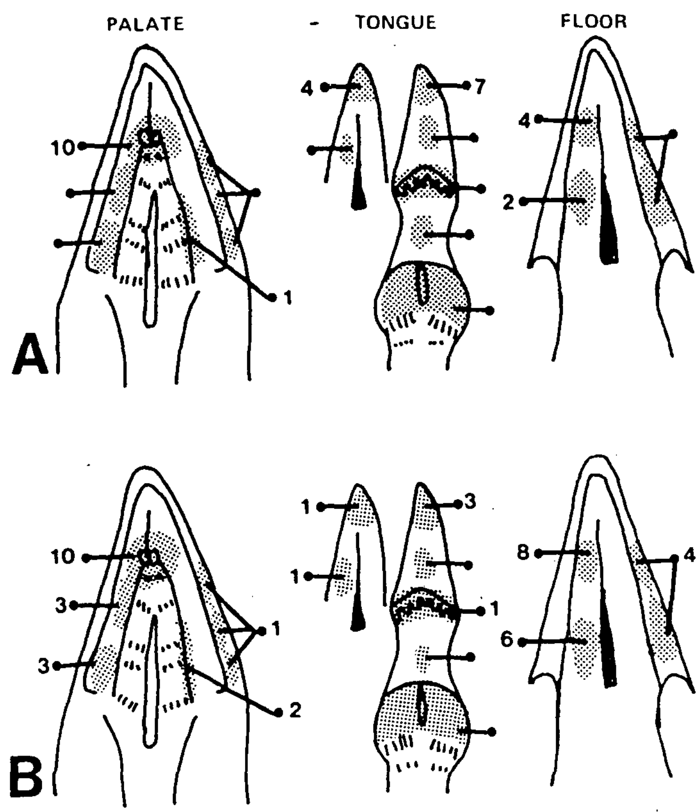
Pale yellow beaks, combs and legs occurred by day 17 in all experimental groups. The feathers were dishevelled and the barbs were disorganised and wavy. Individual feathers, most noticeable in the reminges, had a thin blade because of acute angles formed between the barbs and rachus (Fig.1).

The incidence and severity of oral lesions did not vary with dose and were decreased by day 42. Some lesions resolved while new ones formed. No oral lesions occurred in birds that died before day 4. Yellow plaques occurred first near duct openings of the maxillary salivary gland, tip of the tongue and floor of the buccal cavity (Text-fig.2). Foci and streaks later developed on or lateral to the palatine ridges and on the ventral and dorsal surfaces of the tongue. The beak was more severely affected near the commissures. Yellow plaques covered the blunted lingual papillae. A superficial crust comprised thick gray-yellow exudate mixed with feed and adhered to the underlying ulcerated mucosa (Fig.2).

Necropsy findings in chickens that died included dehydration, yellow, swollen, friable liver with multiple red foci (Fig.3), distended gallbladder, swollen kidneys with white

Table 5. Packed cell volume values of chickens fed Fusarium sporotrichiella *corn cultures.*

Diet % fungal culture	Packed cell volume (%)			x̄ ± S.E. Range
	Trial day			
	7	17	37	42
10%	32 ± 4 28 – 38	31 ± 5 23 – 35		
5%	29 ± 5 23 – 37	26 ± 14 8 – 52		
1%	31 ± 2 27 – 35	31 ± 2 27 – 32	24 ± 8 11 – 33	23 ± 10 6 – 31
0%	33 ± 2 30 – 35	31 ± 1 29 – 32	30 ± 1 29 – 32	31 ± 1 30 – 33



Text-fig. 2. Distribution of Fusarium-*induced oral lesions in broiler chickens fed 5%* Fusarium *diet. (A) Day 7, 10/10 birds affected, (B) Day 17, 10/10 birds affected.*

Note: *Lesions occurred in this study only at the sites marked with numerals, which represent the numbers of birds affected.*

urate precipitates in the ureters, and increased pericardial fluids. Ductular openings were reddened in the proventricular mucosa. The duodenal mucosa was red and the intestinal contents were fluid and yellow.

Many chickens that survived treatment had carcass emaciation. The bone marrow was either pale red or yellow, and some had multiple red foci. The thymus, spleen and bursa of Fabricius were atrophied (Fig.4). White or yellow superficial exudate covered mucosal ulcers in the crop. The gizzard lining was roughened. Intestinal contents were either yellow and fluid or were scanty.

The weights of the spleen, bursa of Fabricius and liver were reduced (Table 4) and differed significantly ($P \leqslant 0.05$) from controls. The reductions in organ weight were related to increases in the dietary concentration of *Fusarium* culture.

Microscopic lesions were in lymphoid and haematopoietic tissues, alimentary tract mucosa, liver and gallbladder, feathers and thyroid glands. Necrosis of lymphocytes was severe in chickens that died by hour 24 (Figs.5, 6) and lymphocyte depletion was severe (Fig.7) in those that died later and in the survivors of 5% and 10% *Fusarium* diets. Consumption of 1% *Fusarium* for 42 days resulted in either mild depletion of lymphocytes or normal lymphoid organs. Germinal centres in the caecal tonsil were reduced in number and sometimes were absent. Large macrophages with eosinophilic foamy cytoplasms were in some caecal tonsils that were depleted of lymphocytes.

The haematopoietic component of bone marrow had necrosis, cell depletion, and macrophages filled with cell debris in both the erythroid and myeloid regions (Fig.8) in the chickens that died. Reductions in the cellularity of the haematopoietic component ranged from mild to severe in the survivors.

Foci of necrosis and ulceration in the buccal mucosa were covered by a crust comprising fibrin, cell debris, keratin, feed particles and bacterial colonies. Necrosis and inflammation extending to the maxillary salivary glands, mandibles and the cartilagenous extensions of the basihyoid bone comprised severe lesions (Figs.9, 10). Many ulcers had a base of granulation tissue containing macrophages and heterophils. The mucosa bordering ulcers was usually thickened and oedematous and contained heterophils. Hyperkeratosis and parakeratosis occurred in the keratinised mucosa near the beak.

Congestion and necrosis of mucosal epithelium and necrosis of lymphocytes of the lamina propria of the proventriculus, ventriculus, and small and large intestine occurred in chickens that died. The ductular epithelium of the proventricular glands had basophilic cytoplasmic bodies 2 to 3 μm in diameter which were interpreted to be apoptotic bodies. Single necrotic cells were detached and in the ductular lumen. Changes in intestinal mucosa included necrosis of epithelium on the villi and in crypts, reductions in the number of mitotic figures in crypt epithelial cells, and mild blunting of villi.

Focal necrosis of hepatocytes and haemorrhages occurred in the liver of chickens that died (Fig.11). Necrosis of intrahepatic bile duct epithelium and biliary casts were additional findings. Basophilic cytoplasmic bodes, 2 to 3 μm in diameter were also in extrahepatic bile duct and gallbladder epithelium (Fig.12). Necrosis and oedema of gallbladder mucosa was seen in one chicken fed 50% *Fusarium* culture. Hepatocytes of surviving *Fusarium*-treated chickens had large cytoplasmic vacuoles that contained lipids (oil red O stain); control livers had smaller droplets. Hyperplasia of bile ductules was mild in *Fusarium*-treated chickens.

Fig. 1. The barbs on the feathers of chickens fed Fusarium *failed to spread causing a narrow blade and they were wavy and disorganised.*

Fig. 2. Yellow crust on the palate and tongue of a chicken fed 5% Fusarium *diet for 17 days.*

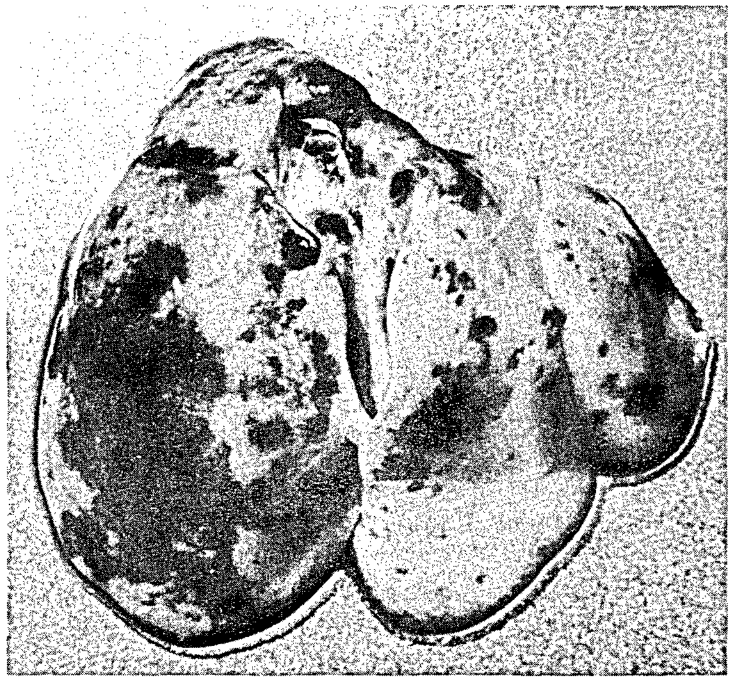


Fig. 3. Haemorrhages in the liver of a chicken fed 25% Fusarium *diet.*

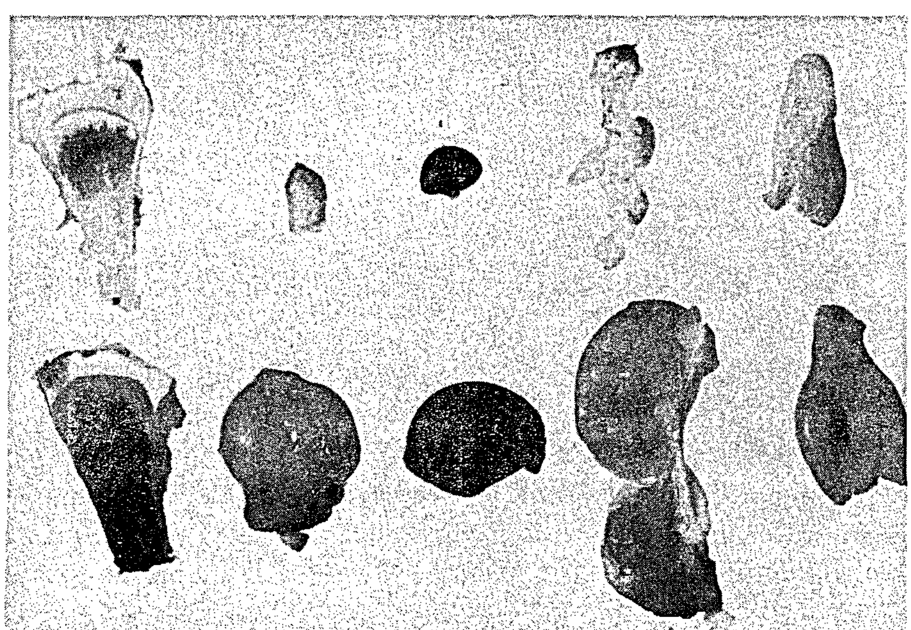


Fig. 4. Pale yellow marrow and atrophied lymphoid organs (top) of a chicken fed 1% Fusarium *diet for 42 days; (bottom) control tissues.*

Fig. 5. Necrosis of cortical and medullary lymphocytes in the bursa of Fabricius of chicken fed 50% Fusarium *diet.*

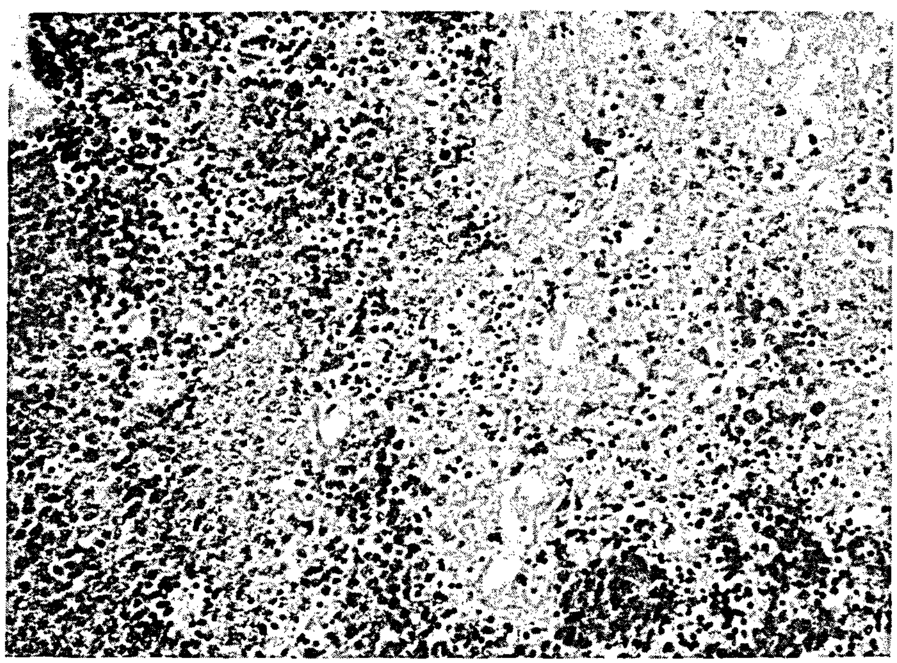


Fig. 6. Necrosis of cortical and medullary lymphocytes in the thymus of a chicken fed 10% Fusarium *diet.*

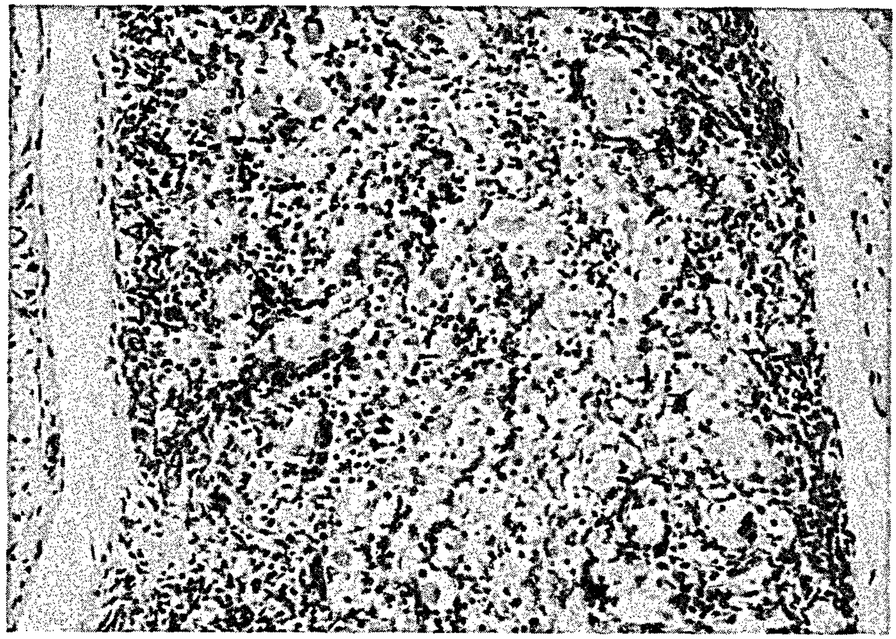


Fig. 7. Severe depletion of lymphocytes in the thymus of a chicken fed 50% Fusarium *diet.*

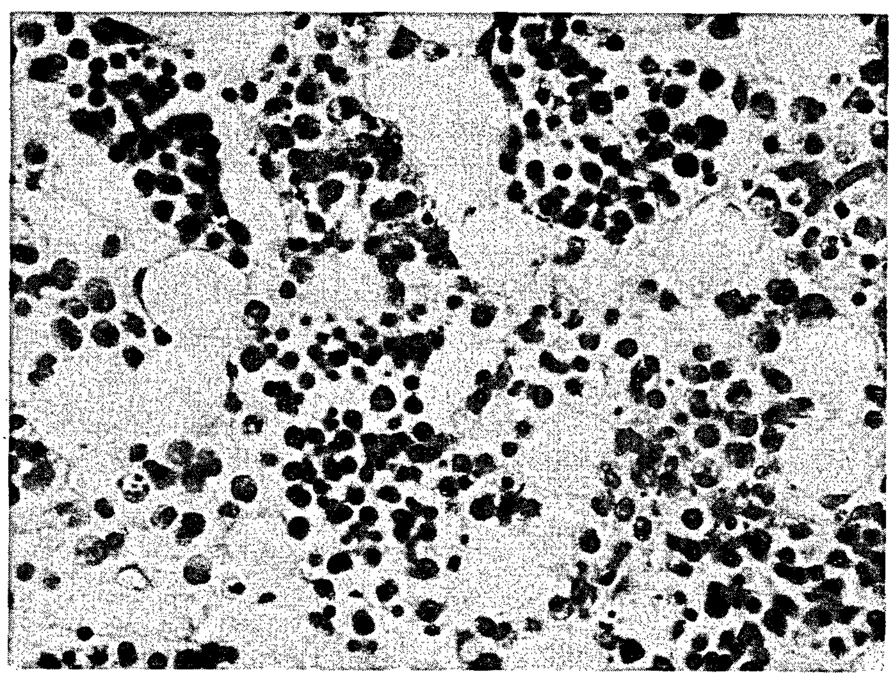


Fig. 8. Necrosis of haematopoietic components of the bone marrow in a chicken fed 50% Fusarium *diet.*

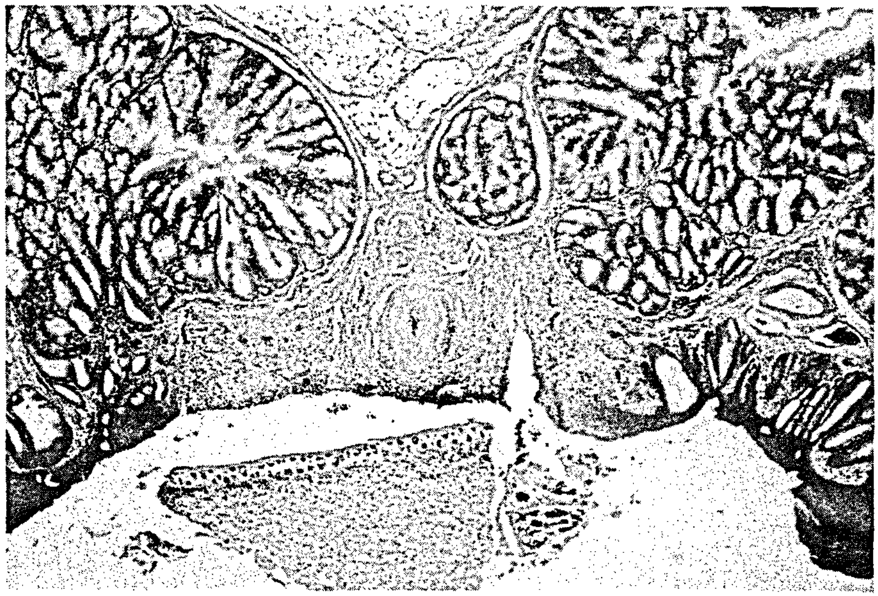


Fig. 9. Ulceration of the oral mucosa between the maxillary salivary duct openings in a chicken fed 1% Fusarium *diet.*

Fig. 10. Necrosis and ulceration of the oral mucosa with necrosis of the maxillary salivary glands in a chicken fed 10% Fusarium *diet for 15 days.*

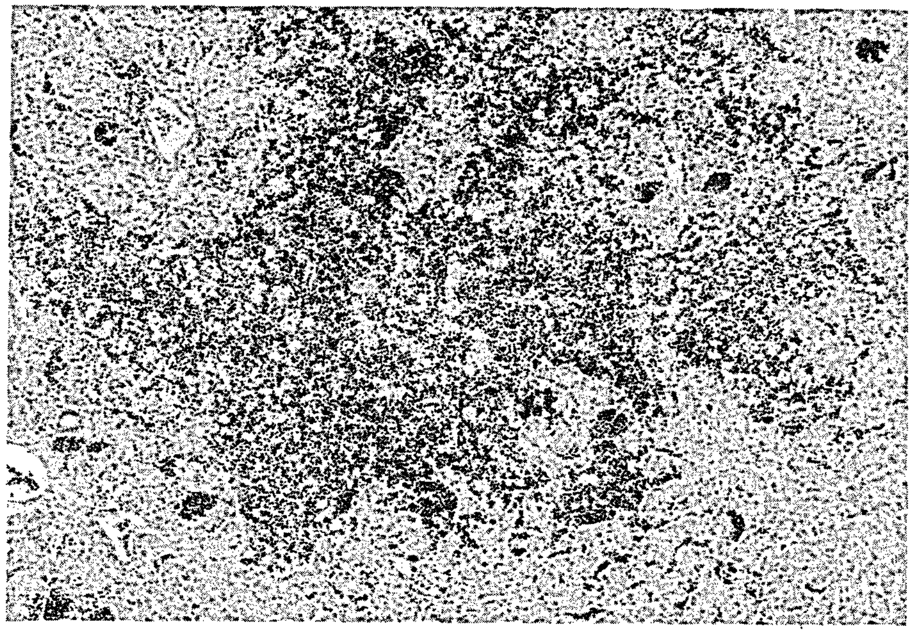


Fig. 11. Focal haemorrhage and necrosis in the liver of a chicken fed 50% Fusarium *diet for 22 hours.*

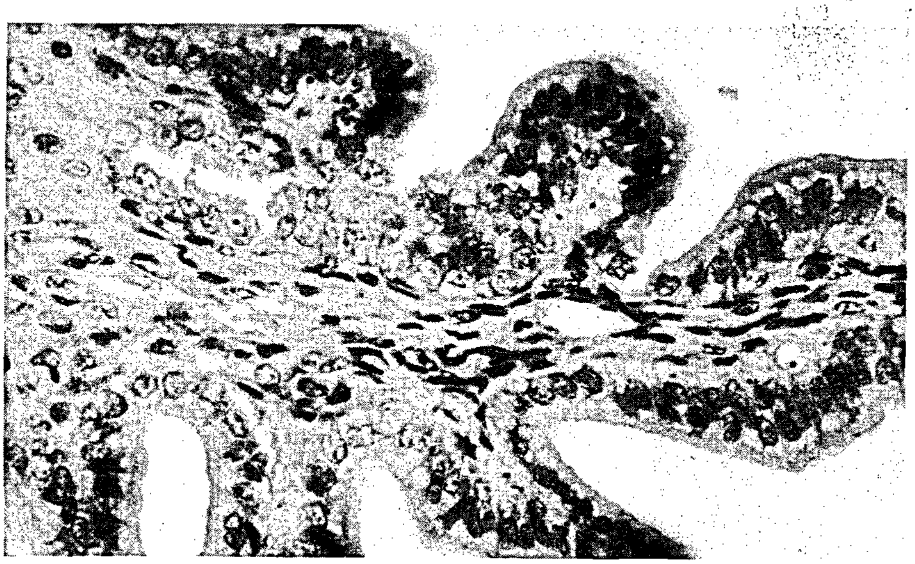


Fig. 12. Basophilic cytoplasmic bodies in the gallbladder mucosal epithelium of a chicken fed 50% Fusarium *diet for 18 hours.*

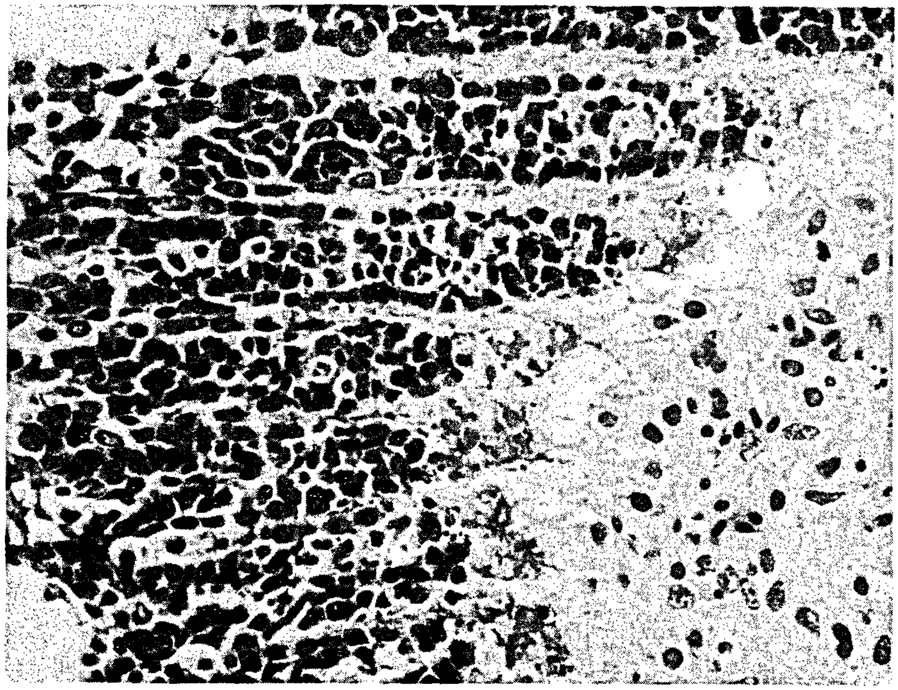


Fig.13. Necrosis of the barb ridges in a feather of a chicken fed 50% Fusarium *diet.*

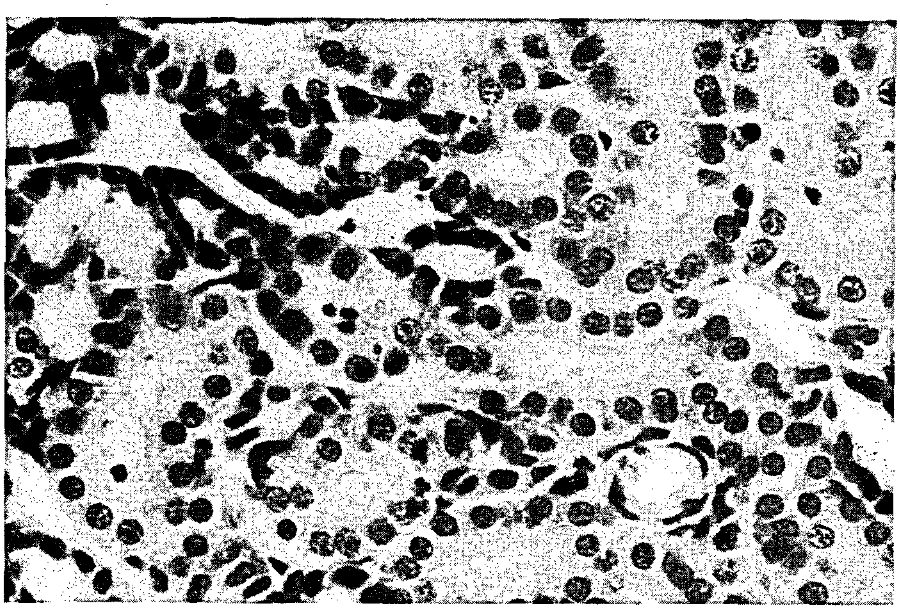


Fig.14. Necrosis of renal tubular epithelium in a chicken given a 5% Fusarium *diet.*

Necrosis of feather epidermis in the feather base, rachus, and *strata basale* and *intermedium* of the barb ridge (Fig.13) occurred in many chickens that died and in a survivor of *Fusarium* treatment. Necrosis of renal tubular epithelium (Fig.14) and interstitial oedema in the kidney occurred with low incidence and mild severity in chickens that died.

The thyroid follicles were small in diameter and the height of follicular epithelium was increased in the survivors of *Fusarium* treatment in comparison to the controls.

The brain, spinal cord, sciatic nerve, heart, lung, adrenal gland, parathyroid gland, and testis also were examined and resembled control tissues.

DISCUSSION

Clinical signs and lesions in this study were similar to those in chickens given either T-2 toxin or diacetoxyscirpenol in the diet or by crop gavage (Hoerr, 1981; Hoerr *et al.*, 1981a, b). One clinical effect that differed in *Fusarium*-treated chickens was an abnormal righting reflex. This transient sign was not associated with either anaemia or lesions in the central or peripheral nervous system, or other target organs. It occurred in chickens that were fed T-2 toxin and which had seizure-type activity in addition (Wyatt *et al.*, 1973). We did not see seizures and the pathogenesis of the altered reflex was not determined.

Acute fusariotoxicosis of chickens could occur as a syndrome of feed refusal and sudden death without the oral lesions that are considered typical of the disease. Depending on toxin concentration in the feed, oral lesions may not occur for 4 days as seen in Trial 1. Necrosis of the liver, lymphoid and haematopoietic tissues, and enteric mucosa, and severe dehydration would be the likely postmortem findings in chickens with acute natural intoxication.

Lesions of oral mucosa are important and the pathoanatomic distribution is a useful diagnostic feature of avian fusariotoxicosis. It would be inappropriate, however, to use oral lesion incidence and severity as a quantitative indicator of either toxin concentrations in the feed or the duration of exposure.

Mycotoxin analysis, conducted after the conclusion of animal studies, revealed that the 8°C fusarial culture contained the least amount of toxin but caused the highest mortality in Trial 1. This apparent contradiction was probably related to feed consumption. Feed refusal was dose-related in chickens given T-2 toxin (Hoerr, 1981). All of the 50% *Fusarium* diets were lethal, but the 8°C culture was probably least refused and a larger dose of toxin was eaten. Refusal of trichothecene-contaminated feed in avian fusariotoxicosis in the field may actually have a sparing effect on mortality.

The combination of calculated concentrations of T-2 toxin (0.5 ppm) and neosolaniol (0.05 ppm), and possibly other undetected toxins, caused marked reductions in weight gain. The "no effect concentration" of T-2 toxin on weight gain was considered to be between 0.2 and 2.0 ppm (Mirocha, 1979). Our data suggested that the lower value would more readily reflect toxic effects of naturally contaminated feedstuffs.

The lethal-dose values determined in this study suggested that the *Fusarium* culture was more toxic than indicated by concentrations of trichothecenes (Table 6). The different durations of the three studies in Table 6 was considered a minor factor because most deaths occurred within 7 days.

Table 6. The toxicity of T-2 toxin given in different forms to broiler chickens.

Treatment	Days	Lethal concentration	Lethal dose (mg/kg body wt/day)	Reference
Crystalline T-2 toxin in diet	7	300 ppm	10.0[a]	Hoerr, 1981
Multiple oral dose T-2 toxin in DMSO: saline (1:9)	14	–	2.9	Hoerr *et al.*, 1981a
Fusarium culture 10% diet	17	5 ppm T-2 toxin plus 0.5 ppm Neosolaniol	0.24[a] 0.02	Present study

a Approximate values calculated from the average daily feed consumption.

One reason for the discrepancy may have been potentiation of toxicity by combinations of toxins. T-2 toxin, neosolaniol and diacetoxyscirpenol were detected in the fusarial cultures and differ structurally only in the grouping (R_3) at the carbon 8 position (Mirocha, 1979). T-2 toxin ($R_3 = (CH_3)_2CHCH_2COO-$) and diacetoxyscirpenol (R_3 = H), given as either single or multiple doses by crop gavage to broiler chickens, had additive lethal effects (Hoerr *et al.*, 1981a). The toxicity of neosolaniol (R_3 = OH) in combination with either T-2 toxin or diacetoxyscirpenol has not been measured, but in this study, neosolaniol might have potentiated the toxicity of T-2 toxin. Other fusarial toxins such as zearalenone or butenolide also could have influenced the toxicity of trichothecenes. We did not assay for zearalenone, but butenolide was not present at the 25 ppm detection limit. Other components of the fusarial culture which could have potentiated the toxicity of trichothecenes remain unknown.

Another possible influence, refusal of feed, was not a factor in the toxicity of T-2 toxin to haematopoietic and lymphoid tissues (Hoerr, 1981). The effect of nutritional differences in the experimental and control diets was slight because diets with only 1% whole culture were toxic.

The toxicity of pure T-2 toxin added to diets was similar to that of the toxins in either whole cultures of *Fusarium* or the extract when given to calves and pigs (Patterson *et al.*, 1979) and laying hens (Speers *et al.*, 1977), but re-evaluation of other published data reveals possible discrepancies. An exposure to T-2 toxin of 30 mg/cow/day was estimated for a lethal intoxication of dairy cattle caused by *Fusarium tricinctum*-moulded corn (Hsu *et al.*, 1972). This would have been a daily dose 0.06 mg/kg body weight for a 500-kg dairy cow. This should have had little effect because a dairy cow that was given pure T-2 toxin in daily doses of 0.44 mg/kg developed only mild diarrhoea (Weaver *et al.*, 1980). T-2 toxin was lethal for chickens when given as extracts of fusarial cultures in multiple oral doses of 0.35 to 1.2 mg toxin/kg body weight (Joffe and Yagen, 1978). These values, however, are less than half the multiple-oral dose LD_{50} (2.9 mg/kg) of T-2 toxin for chickens (Hoerr *et al.*, 1981a).

Definition of the toxicity of combinations of trichothecenes as a group (Ciegler, 1978;

Smalley *et al.*, 1977) and with other fusarial toxins could resolve these apparent conflicts in toxicity data which exist especially for chickens. The toxicity values of purified trichothecenes should be applied cautiously in evaluation of the generally low concentrations of trichothecenes found in naturally contaminated feedstuffs (Smalley, *et al*, 1977; Mirocha, 1979).

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RESUME

Mycotoxicose expérimentale à Trichothècene produite chez le poulet de chair par *Fusarium sporotrichiella* var. *sporotrichioides*

Fusarium sporotrichiella var. *sporotrichioides* (Bilay), cultivée sur du popcorn stérile à 23°C, et ensuite à 8, 16 et 23 degrés et administrée à raison de 50% du régime alimentaire a été létal pour des poussins de chair mâles âgés de 7 jours. La culture à 8°C contenant de la toxine T-2 à 50 parties par million (ppm) et du néosolaniol à 5 ppm a été donnée en culture totale à des concentrations de 10%, 5%, 1% et 0% pendant 17 jours ainsi que 1% pendant 42 jours. La moitié des poulets qui avaient reçu le régime à 10% sont morts au cours des 17 jours (5 ppm de toxine T-2 et 0,5 ppm de néosolaniol). La dose correspondante journalière était de 0,22 mg de toxine T-2 et 0,02 mg de néosolaniol par kilo de poids vif et par jour. Les poulets qui mourraient étaient déshydratés, avaient une nécrose et une déplétion des tissus lymphoïdes et hématopoiëtiques ainsi qu'une nécrose du système hépatobiliaire, de la muqueuse gastro-intestinale de l'épiderme plumeux et de l'épithélium tubulaire rénal. Les survivants présentaient une anémie, une réduction du gain de poids et une altération transitoire des réflexes. La crête et le bec étaient jaune pâle et les barbes des plumes ébouriffées. Les survivants avaient aussi une atrophie des tissus lymphoïdes, une réduction des cellules hématopoiëtiques dans la moëlle osseuse, une nécrose de la muqueuse de la bouche et du jabot, des hépatocytes vacuolisés, une hyperplasie des canalicules biliaires et une réduction du diamètre des follicules thyroïdiens.

ZUSAMMENFASSUNG

Experimentelle Trichothecene Mycotoxikose bei Broilerküken durch *Fusarium sporotrichiella* var. *sporotrichoides*

Fusarium sporotrichiella var. *sporotrichioides* (Bilay), der auf sterilisiertem Popkorn bei 23°C und dann anschließend bei 8°C, 16°C und 23°C kultiviert worden war, das dann als 50%-iger Futteranteil verfüttert wurde, erwies sich als lethal für 7 Tage alte

männliche Broilerküken. Die 8°C-Kultur, die 50 ppm T-2-Toxin und 5 ppm Neosolaniol enthielt, wurde als Gesamtkultur zu 10%, 5%, 1% und 0% 17 Tage lang dem Futter beigemischt und außerdem zu 1% während 42 Tagen. Die Hälfte der Küken, die das 10%-ige Futter (5 ppm T-2 Toxin und 0,5 ppm Neosolaniol) erhielten, starben innerhalb von 17 Tagen. Die entsprechende Tagesdosis betrug 0,22 mg T-2 Toxin und 0,02 mg Neosolaniol/kg Kgw./Tag. Die verendeten Küken waren dehydriert, und zeigten Nekrosen und eine Zellentspeicherung der lymphatischen und haematopoietischen Gewebe und Nekrosen im Hepatobiliarensystem, in der Magendarmschleimhaut, in der Epidermis und im Tubulusepithel der Nieren. Die überlebenden Tiere wiesen eine Anaemie, eine Reduktion der Gewichtszunahme und eine vorübergehende Änderung des Streck-Reflexes auf. Kamm und Schnabel waren blaßgelb und die Federfahnen waren zerzaust. Die Überlebenden zeigten eine Atrophie der lymphatischen Gewebe, eine Reduzierung der blutbildenden Zellen im Knochenmark, Nekrosen in Mund- und Kropfschleimhaut, vakuolisierte Hepatozyten, hyperplastische Gallengänge une eine Reduktion der Follikeldurchmesser in der Schilddrüse.